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Association of vitamin D intake and serum levels with fertility: Results from the Lifestyle and Fertility Study

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Abstract

Objective—To evaluate the role of vitamin D intake and serum levels on conception of clinical pregnancy and live birth.

Design—Prospective cohort study.

Setting—Academic medical centers.

Patient(s)—Healthy, nulliparous women, aged 18-39 years and their male partners.

Intervention(s)—None.

Main Outcome Measure(s)—Clinical pregnancy and live birth were compared between those who did or did not meet the vitamin D Estimated Average Requirement (EAR) intake (10 μg/d), and with serum 25(OH)D considered at risk for inadequacy or deficiency (<50 nmol/L) or sufficient (≥ 50 nmol/L).

Results—Among 132 women, 37.1% did not meet the vitamin D EAR and 13.9% had serum levels at risk for inadequacy or deficiency. Clinical pregnancies were significantly higher among women who met the vitamin D EAR (67.5% vs. 49.0%) and with sufficient serum 25(OH)D (64.3% vs. 38.9%) compared to those who did not. Live births were higher among those who met the vitamin D EAR (59.0% vs. 40.0%). The adjusted odds ratio (AOR) of conceiving a clinical pregnancy was significantly higher among those who met the EAR (AOR: 2.26, 95% CI

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1.05-4.86) and had sufficient serum 25(OH)D (AOR: 3.37, 95% CI 1.06-10.70). The associations were not significant after controlling for selected nutrients and dietary quality.

Conclusions—Women with vitamin D intake below EAR and serum 25(OH)D levels at risk for inadequacy or deficiency may be less likely to conceive and might benefit from increased vitamin D intake to achieve adequacy.

Clinical Trial Registration Number: NCT00642590

Keywords

vitamin D; serum 25(OH)D; pregnancy; fertility

Introduction

Vitamin D is a fat soluble vitamin that functions to maintain calcium and phosphorus homeostasis and promote bone mineralization (1). Sources from food and supplements, and sun exposure provide the inert vitamin D2 (ergocalciferol) and D3 (cholecalciferol) forms which undergo a two-step hydroxylation process to produce 25-hydroxyvitamin D [25(OH)D] in the liver and the physiologically active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in the kidneys (1). In the US, about one-quarter of the population is at risk of inadequacy or deficiency in vitamin D as measured by levels of total serum 25(OH)D (2). Recent studies suggest that vitamin D status may have an impact on fertility, pregnancy, and birth outcomes (3-16). Biological actions of vitamin D are mediated through vitamin D receptors (VDR) that are distributed across various tissues (17-21). The presence of VDR in reproductive tissues in both males and females suggests a possible role in the regulation of reproductive processes. In females, VDR are expressed in the ovaries, placenta, and endometrium (17-19). In males, VDR can be found in smooth muscles of the genital tract, testicular tissue, and sperm (20-21).

Animal studies have shown that vitamin D deficiency reduces mating success and fertility (3, 22-23). In vitamin D deficient male rats, there is a reduction in successful mating, incomplete spermatogenesis, degenerative changes, low sperm counts and reduced number of mobile spermatozoa (3, 24-25). Studies of compromised VDR expression in female rats have reported impaired folliculogenesis, uterine hypoplasia, infertility, and pregnancy complications (3, 26-27).

In observational studies of men, decreased vitamin D levels have been associated with poor semen quality, low sperm count, and abnormal motility and morphology (3, 28) The function of vitamin D in male fertility is not known, but may be related to the regulation of calcium (3, 29). In women, observational fertility-related research has focused primarily on endometriosis and polycystic ovarian syndrome (PCOS) (3, 30-33). Data from observational studies suggest that the vitamin D regulatory network is involved in the pathogenesis of endometriosis through the dysregulation and over expression of VDR in the endometrium, and vitamin D deficiency is associated with insulin resistance and PCOS (30-33).

In studies that have focused on infertility and assisted reproductive technology treatments, 25(OH)D levels in serum and follicular fluid have been measured and compared with IVF

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success (3, 6, 8-10, 34-36). A recent study showed that serum and follicular fluid concentrations of 25(OH)D were highly correlated with success in achieving a clinical pregnancy (9). In another study, however, while women with higher serum 25(OH)D levels had higher fertilization rates there was no association between serum level and chance of a clinical pregnancy and live birth (36).

Studies of the role of vitamin D in conception among healthy women are lacking; only one study has examined pre-conception plasma 25(OH)D levels and the probability of subsequent pregnancy (11). In that study, plasma concentrations of 25(OH)D were not associated with the chance of conceiving. To date, no studies have assessed the impact of dietary and supplemental vitamin D intake on conception success. In the current study, we prospectively evaluated the association between dietary and supplemental vitamin D intake and serum 25-hydroxyvitamin D levels and the probability of conceiving a clinical pregnancy and having a live birth, and the time to conception among couples who were planning their first pregnancy.

Material and Methods

Subjects

Subjects were participants in the Lifestyle and Fertility Study (ISIS Study), a prospective cohort study of healthy, nulliparous couples with no known infertility conditions, who were planning their first pregnancy (37). The overall objective of the study was to examine the association between antioxidant status and pregnancy. To be eligible, women must have been having regular menstrual periods and using contraception at the time of enrollment. Women were excluded if they had a previous recognized conception, history of attempting to conceive, diagnosis of PCOS or endometriosis, or any serious medical condition such as cancer, heart disease, or diabetes. One hundred and thirty-two women between the ages of 18-39 years and their male partners were enrolled between May 2006 and June 2012 from three geographic areas: Boston, MA (42°N), University Park, PA (41°N), and Lebanon, NH (44°N). The protocol was approved by the human subjects review boards at all the participating institutions and all participants provided written informed consent.

Study Protocol

Lifestyle factors, vitamin D intake, and serum measures of vitamin D status were assessed in all participants. Urine samples starting on the first day of the first menstrual cycle after the baseline visit were assayed for daily measures of human chorionic gonadotropin (hCG). Couples were followed from the start of the study protocol until they conceived a clinically confirmed pregnancy, completed six menstrual cycles of attempted conception, or dropped out. Those who conceived a clinical pregnancy were followed through its outcome. Those who did not conceive within six menstrual cycles were contacted at 12 months to determine whether they had become pregnant.

Vitamin D Intake

Vitamin D intake was assessed using a series of three, unannounced, telephone 24-hour dietary recalls prior to conception at baseline (0-month) and again at 3 and at 6 months if couples had not achieved a clinically confirmed pregnancy. Dietary recalls were conducted by trained interviewers at The Pennsylvania State University Diet Assessment Center (University Park, PA). A standardized process including the multiple-pass technique was utilized (38). Quantity and portion sizes consumed were ascertained with the assistance of a portion size guide provided to the participants. Dietary recall included all foods, beverages and supplements consumed by the participants over the previous 24-hour period and included two weekdays and one weekend day. Intakes were averaged among the three recall days. To reflect the marketplace throughout the study, dietary intake data were collected using Nutrition Data System for Research (NDSR) software versions 2008, 2009, 2010, and 2011 developed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN. Final calculations were completed using NDSR version 2012. The NDSR time-related database updates analytic data while maintaining nutrient profiles true to the version used for data collection. Dietary intake was adjusted for total energy intake using the nutrient residual method to reduce extraneous variation in non-energy-bearing nutrients (39). In this method, the nutrient is regressed on the total energy intake and a residual is computed in which total energy intake is the independent variable and the absolute nutrient intake is the dependent variable.

We examined three categories of nutrient intake: dietary nutrient intake (diet sources only), dietary supplements alone, and total nutrient intake (both dietary and supplement sources). Intake closest to conception was used in the analysis as follows: 0-month intake for those who conceived during the first three menstrual cycles, 3-month intake for those who conceived during the next three menstrual cycles, and 6-month intake for those who did not conceive by the end of the study protocol. Vitamin D intake was compared to the Institute of Medicine's (IOM) Estimated Average Requirement (EAR), 10 μg/d, the daily intake needed to meet the requirement in half of all women and men ages 19-50 years (1). The EAR comparison groups were determined by using total vitamin D intake from food and supplements.

Serum Vitamin D

At baseline (0-month), fasting blood samples (7mL) were collected and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpms for 20-30 minutes, processed, and stored at -80°C. Serum 25(OH)D was analyzed at the Nutritional Biomarkers Branch of the Centers for Disease Control and Prevention using standardized liquid chromatography tandem mass spectrometry (LC-MS/MS) method (2). This method is traceable to international reference materials. The laboratory participates in several quality assurance programs and uses multiple levels of quality control materials in every assay. Total 25(OH)D is defined as the sum of 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3. The method separates 25-hydroxyvitamin D_3 from the C3-epimer of 25-hydroxyvitamin D_3, which is excluded from the total 25(OH)D concentration. Measures that were below the limit of detection were assigned a value that was calculated as the lower limit of detection divided by the square root of two, as employed in the National Health and Nutrition Examination Survey (NHANES). The lower limit of detection for 25-hydroxyvitamin D_2 was 2.05 nmol/L (n=59 for females and n=61 for males), 25-hydroxyvitamin D_3 was 2.23 nmol/L (n=0 for both females and males). Vitamin D status was determined as either sufficient, or at risk for inadequacy or deficiency when serum 25(OH)D levels were ≥ 50 nmol/L or <50 nmol/L, respectively. Serum 25(OH)D ≥ 50 nmol/L, as recommended by the IOM, is recognized to be adequate for bone health in nearly all healthy individuals (1).

Covariates

Demographic and lifestyle characteristics including age, race, education, income, smoking status, alcohol consumption, and prior contraceptive use were reported by participants at baseline. Physical activity, both aerobic and low intensity, was ascertained from the participants at baseline. Physical activity was then scored as high or low based on the frequency and type of activity according to the US Department of Health and Human Services recommended Physical Activity for Americans guidelines (40). In addition, body weight and height were measured by study staff according to the NHANES protocol, and body mass index (BMI) (kg/m^2) was calculated for each participant (41). BMI categories were created using the National Heart, Lung, and Blood Institute recommended BMI and weight status ranges as follows: underweight (BMI <18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (≥ 25 to <30 kg/m^2), and obese (≥ 30 kg/m^2) (42).

Energy adjusted intakes of selected nutrients including polyunsaturated fat, iron, and folic acid from diet and supplements were examined as covariates. The Alternative Healthy Eating Index for Pregnancy (AHEI-P), a modified version of the Alternative Healthy Index (AHEI), was used to assess overall dietary quality (43-46). The AHEI, an *a priori* dietary index based on the Healthy Eating Index developed by the US Department of Agriculture, measures diet quality, including assessment of nutrients associated with decreased chronic disease risk (45-47). AHEI-P is modified for pregnancy to focus on dietary components that include nutrients important during pregnancy (i.e., folate, and iron) (43-44).

Pregnancy Outcomes

Pregnancy was first determined by positive pregnancy test (hCG ≥ 20 mIU/ml; AIM MidStream OTC Professional, Craig Medical Distribution, Inc.) and confirmed clinically. Each confirmed pregnancy was followed to determine its outcome. The date of fetal loss or delivery of a live birth was recorded. Live birth was defined as a gasp, heartbeat, or sign of life at birth. Time to conception of a clinical pregnancy was defined as the length of time from the first day of the menstrual period following baseline visit, when the couple began attempting conception, to the date of the last menstrual period plus 14 days. Time was censored at the date of baseline visit for those who became pregnant prior to the start the urine collection protocol. For those who did not conceive, time was censored at the end of six menstrual cycles or on the last day of urine collection if the daily urine collection protocol was not completed for six menstrual cycles.

Statistical Analysis

Descriptive statistics for continuous variables are presented as means ± SD or geometric means (95% confidence intervals) for those variables that were back transformed after use of natural log transformations during data analyses (vitamin D intake), and percentages for categorical variables. The percentage of men and women who did or did not meet the EAR for vitamin D intake was determined. Sufficiency of serum 25(OH)D levels was categorized into two groups: those with sufficient levels and those with levels that were with at risk for inadequacy or deficiency. Intakes between the groups meeting or not meeting the EAR for vitamin D were compared using two-sample t-tests. Differences in clinical pregnancy and live birth outcomes were assessed using the chi-square test, Fisher's Exact test and multiple logistic regression to calculate odds ratios with 95% confidence intervals. Age, race, BMI category, alcohol consumption, and smoking status were controlled for in the first adjusted model for vitamin D intake. A second adjusted model included total energy intake, total dietary and supplement energy adjusted intakes of polyunsaturated fats, iron and folic acid, and AHEI-P score. For serum 25(OH)D, age, race, BMI category, alcohol consumption, smoking status, and physical activity (proxy for sun exposure) were controlled for in the first model. Model 2 included total energy intake, total dietary and supplement energy adjusted intakes of polyunsaturated fats, iron and folic acid, and AHEI-P score. Time to conception was analyzed using a Cox proportional hazards model. Cumulative incidence of time to conception was plotted as one minus Kaplan-Meier estimates. Statistical significance was defined as $P < 0.05$ (two-sided). Data analyses used SAS 9.2 statistical software (SAS Institute Inc., Cary, NC).

Results

The study population comprised 132 female participants and 131 male partners who enrolled in the study. Included in the analyses were all subjects who completed dietary recalls (n=132 females and n=129 males) and provided samples for blood collection in which serum vitamin D levels were measurable (n=130 for both females and males). Baseline demographic and clinical characteristics, and selected dietary intake measures stratified by sex and EAR category for vitamin D intake are presented (Table 1). Household income (P=0.002), completion of college or advanced degree (P=0.01), total iron (P<0.0001) and folic acid (P<0.0001) intakes from diet and supplements were higher among women who met the vitamin D EAR compared to those who did not. Men who met the EAR were less racially diverse (all Caucasian; P=0.006), had lower body mass index (P=0.04) and were less likely to be obese (P=0.01). Men who met the EAR were also more likely to have higher intakes of energy (P=0.01), iron (P=0.0003), and folic acid (P<0.0001), as well as a higher AHEI-P score for dietary quality (P=0.002). No differences in any other demographic or lifestyle characteristics were observed in either sex when compared by EAR category.

Overall, 96% of the women did not meet the EAR for vitamin D through diet; average intake was 4.4 μg/d from food sources alone (Table 2). The average total vitamin D intake increased to 16.5 μg/d when vitamin D containing supplements were considered together with dietary intake. While there was an increase in intake provided by the vitamin supplement, 37% of women remained below the EAR for vitamin D. Similarly, the men had an average vitamin D intake of 4.5 μg/d from food sources alone with 94% not meeting the EAR. The number of men using a vitamin D containing supplement was lower than the women and even with supplement use, 68% of the men were still below the EAR for total vitamin D intake, with an average total intake of 12.4 μg/d. When examining the intake by couple, 91% of the couples were both below the EAR for vitamin D intake from foods alone and 30% for total vitamin D intake from food and vitamin supplements (Data not shown).

Total vitamin D intake was significantly higher among women who met the EAR (17.5 µg/d) compared to 4.6 µg/d among those who did not meet the EAR (Table 2). Among men, supplement intake and total intake were also significantly lower among those who did not meet the EAR compared to those who met the EAR. Those who met the EAR had a total intake of 17.9 µg/d compared to 4.1 µg/d among those who did not meet the EAR.

The mean (±SD) serum 25(OH)D levels for both women (70.6 ± 20.7 nmol/L) and men (58.8 ± 18.5 nmol/L) were above 50 nmol/L, the minimum level of sufficiency. Among women, 13.9% had levels indicating inadequacy or deficiency (<50 nmol/L). A greater percentage of men (32.3%) had inadequate or deficient vitamin D status. (Data not shown.)

There were 80 confirmed clinical pregnancies and 69 live births in the study. Those who had a clinical pregnancy had a slightly higher mean vitamin D intake at 11.5 µg/d (95% CI 9.7-13.6 µg/d) compared to those who did not conceive (9.9 µg/d, 95% CI 7.6-12.9 µg/d), though not statistically significant (P=0.33). (Data not shown.) Similarly, mean vitamin D intake was not significantly different between those who had a live birth and those who did not have a live birth (11.7 µg/d [95% CI 9.7-14.1] vs. 9.9 µg/d [95% CI 7.9-12.4], respectively, P=0.25). (Data not shown.) Additionally, there was no association between vitamin D intake as a continuous measure and the odds of conceiving a clinical pregnancy (OR: 1.26, 95% CI 0.79-2.02, P=0.33) or live birth (OR: 1.31, 95% CI 0.82-2.07, P=0.25). (Data not shown.)

When vitamin D intake was categorized by the EAR, women who met the EAR for vitamin D intake had significantly higher percentages of clinical pregnancies (67.5% vs. 49.0%, P=0.036) and live births (59.0% vs. 40.0%, P=0.043) compared to those who did not meet the EAR. Among women who met the vitamin D EAR, the crude odds ratios (OR) were significantly higher for conceiving clinical pregnancies (OR: 2.16, 95% CI 1.05-4.46, P=0.037) and live births (OR: 2.09, 95% CI 1.02-4.29, P=0.044) compared to those who did not meet the EAR. Table 3 presents the adjusted odds ratios (AOR) of conceiving clinical pregnancies and live births by vitamin D intake among women. In model 1, after controlling for age, race, BMI category, alcohol consumption, and smoking status, the association remained significant with an AOR of 2.26 (95% CI 1.05-4.86, P=0.038) for conceiving a clinical pregnancy and for a live birth was 2.20 (95% CI 1.03-4.69, P=0.042) for women who met the EAR compared to women who did not. When considering the additional selected nutrients and overall dietary quality in model 2, this relationship became non-significant for clinical pregnancy (AOR 1.52, 95% CI 0.56-4.15, P=0.42) and live birth (AOR 1.74, 95% CI 0.66-4.60, P=0.26).

When examining mean (± SD) serum 25(OH)D values, there were no significant differences between those who conceived a clinical pregnancy (72.9 ± 20.3 nmol/L) and those who did not have a clinical pregnancy (67.0 ± 20.9 nmol/L, P=0.11). (Data not shown.) Similarly, mean (±SD) serum 25(OH)D levels were 73.4 ± 20.6 nmol/L among those who had a live birth and 67.4 ± 20.5 nmol/L for those who did not (P=0.10). (Data not shown.) There was no association between serum 25(OH)D as a continuous measure and the odds of conceiving a clinical pregnancy (OR: 1.01, 95% CI 0.99-1.03, P=0.12) or live birth (OR: 1.02, 95% CI 0.99-1.03, P=0.10). (Data not shown.)

When examining serum 25(OH)D by sufficiency levels, women with sufficient serum 25(OH)D levels were significantly more likely to conceive a clinical pregnancy than those who were inadequate or deficient (64.3% vs. 38.9%, *P*=0.041) with an OR of 2.83 (95% CI 1.02-7.87, *P*=0.047). Similarly, though not statistically significant, 56.3% of those with sufficient serum levels conceived a live birth compared to 33.3% who were inadequate or deficient (*P*=0.07) with an odds ratio of 2.57 (95% CI 0.90-7.34, *P*=0.078). After adjusting for age, race, BMI category, alcohol consumption, smoking status, and physical activity in model 1, those who had sufficient serum 25(OH)D continued to have a significantly greater chance of conceiving a clinical pregnancy (AOR: 3.37, 95% CI 1.06-10.70, P=0.039). The statistical significance diminished after further adjusting for additional selected nutrients and overall dietary quality in model 2 (Table 3).

When taking into consideration of the intake both the female and male partners together, the couples who met the EAR for total vitamin D intake were significantly more likely to conceive a clinical pregnancy than the couples who did not meet the EAR (67.8% vs. 46.2%, *P*=0.02; OR=2.45, 95% CI 1.14-5.30, *P*=0.02). There was a suggestion that couples who both met the EAR were more likely to have a live birth (57.8% v. 43.6%; *P*=0.14). (Data not shown).

Time to conception for clinical pregnancy was more favorable among women who met the EAR for vitamin D intake, although not statistically significant, (HR=1.19, 95% CI=0.74-1.92, *P*=0.48) with a median time to conception of 3.0 months for women who met the EAR compared to 4.7 months for those who did not. While not statistically significant, women with sufficient 25-hydroxyvitamin D serum levels were more likely to conceive within the first 3 months after baseline (HR=1.60, 95% CI=0.66-3.82, *P*=0.30) [Figure 1].

Discussion

This is the first study that has prospectively examined vitamin D levels from food and supplemental intake and serum 25(OH)D among healthy couples planning their first pregnancy. We report a positive association between total vitamin D intake and the chance of conceiving a clinical pregnancy or live birth. The inability to meet the vitamin D EAR by food intake alone was common among participants with almost all women and men not meeting the recommendations. Use of supplements increased total vitamin D intake, though about 37% of women and 68% men were still unable to meet the vitamin D EAR. We found that women not meeting the vitamin D EAR were less likely to conceive a clinical pregnancy and live birth compared to those who did meet the vitamin D EAR. The odds of conceiving remained significant after controlling for demographic and clinical characteristics. However, after taking into consideration selected nutrient intakes and dietary quality, the statistical significance diminished. When examining the intakes of the female and male together, the results continued to reveal the trend of lower clinical pregnancy rates among those couples who did not consume intakes that met the vitamin D EAR.

In examining serum 25(OH)D, about 14% of females and 32% of males had serum levels that were indicative of being at risk for inadequacy or deficiency. Women with 25(OH)D serum levels that were at risk for inadequacy or deficiency were significantly less likely to conceive a clinical pregnancy than those with sufficient levels, and with a borderline significance observed for live birth when controlling for demographic and clinical characteristics. After adding total energy intake, total dietary and supplement energy adjusted intakes of polyunsaturated fat, iron and folic acid, and dietary quality, the odds of conceiving a clinical pregnancy or live birth were no longer significant.

While mean intake did not differ between those who conceived a clinical pregnancy and live birth, an association was seen between intake and clinical pregnancy and live birth outcomes when intake was examined by the recommended EAR level. We found that intake below the recommended EAR level was associated with lower chances of conceiving clinical pregnancies and live births. The vitamin D EAR set at 10 µg/d is based on the requirement needed to maintain bone health (1). Our finding suggests that intake at the recommended EAR level may perhaps be a threshold at which vitamin D may also biologically impact reproductive health. In the present study, the lower tertile of vitamin D intake was right below the EAR at 9.1 µg/d. When examining intake by tertiles, the upper and middle tertiles compared to the lower tertile had higher percentages of women conceiving clinical pregnancies (63.6%, 70.5% vs. 47.7%) and live births (59.1%, 59.1% vs. 38.6%). Furthermore, when examining supplement use and its contribution to total intake, it can be seen that supplement use helps attain total intake at or above the vitamin D EAR. Our study suggests that there may be a potential for a subgroup population with vitamin D intake below the EAR recommendation that would benefit from an increased intake and supplement use to improve overall vitamin D intake and fertility.

When considering the intakes of the couple as a unit, couples in which both partners met the EAR for total vitamin D intake were significantly more likely to conceive a clinical pregnancy than the couples who did not meet the EAR. Interestingly, when examining the relationship among the male partners individually, men who did not meet the vitamin D EAR were less likely to have female partners who also met the vitamin D EAR than those who did (55.7% vs. 80.5%, P=0.006). Among men who did not meet the vitamin D EAR and had female partners who did meet the EAR, their odds of conceiving a clinical pregnancy were significantly higher than those with female partners who did not meet the vitamin D EAR (OR: 2.78, 95% CI 1.01-7.04, P=0.031) and with live birth (OR: 2.68, 95% CI 1.01-6.75, P=0.031). These results demonstrate the potential impact of intakes of respective partners on conception.

This study obtained high quality dietary intake data and biomarkers utilizing gold standard measures including use of 3-day diet recalls that assessed a representative average of short term dietary intake among participants, and the use of a comprehensive assay that measures 25(OH)D metabolite concentrations with high sensitivity and excellent accuracy. We collected prospective dietary intake data with repeated measures, which allowed us to observe any changes and to determine intakes that reflected the time period closest to conception. There is no common definition for vitamin D deficiency and multiple institutes and societies offer guidelines on vitamin D supplementation. Other fertility studies that have examined serum 25(OH)D levels have used a higher level to classify sufficiency at > 70 nmol/L. The IOM guidelines state a lower threshold of 50 nmol/l for sufficiency (1). We utilized the recommended EAR intake levels and categorization of sufficiency for serum

25(OH)D levels that are defined by the IOM as they are based on well-established evidence of the primary role of vitamin D in supporting the human requirements of bone health in the general population (1). Our vitamin D intake and serum biomarker levels are similar to the NHANES data and are representative of the general population (2).

Our results are in contrast to the only other study to evaluate preconception serum levels of vitamin D in healthy women (11). Like Moller et al., we did not observe significant differences in mean serum between women who conceived and those who did not. However, we saw significantly greater crude odds of achieving a clinical pregnancy for those women with 25(OH)D ≥50 nmol/L and adjusted odds remained statistically significant after controlling for demographic and clinical characteristics (11). The association between serum 25(OH)D and conceiving a clinical pregnancy was no longer significant after adjusting for selected nutrient intake and overall dietary quality. Our categorization of 2 groups for 25(OH)D status rather than 3 groups might have afforded our study a better opportunity to observe the biological impact of low serum 25(OH)D levels on clinical pregnancy outcome. While studies measuring 25(OH)D in serum and follicular fluid in the infertility population undergoing IVF treatment have been inconsistent, some studies have shown similar positive associations between 25(OH)D levels and conception when categorizing patients as vitamin D deficient or replete (10, 34-35).

The study sample size was modest and the numbers that were categorized as at risk for inadequacy or deficiency were limited. This may have hindered us from observing statistically significant differences in achieving live birth. Due to the small sample size, we chose not to model our analyses after the NHANES data by further categorizing those who were at risk for inadequacy (25(OH)D 30-50 nmol/L) or at risk for deficiency (<30 nmol/L) to observe whether there was a difference in fertility among those who were sufficient, at risk for inadequacy, or at risk for deficiency. While our demographic data are consistent with the general US population which is predominantly Caucasian, we did not have the ability to examine impact of demographic and other diverse lifestyle factors on vitamin D intake and serum 25(OH)D levels. Though our study had repeated measures of dietary data, we only measured serum at baseline. However, there were no changes in vitamin D intake over time from baseline to month 3 among those who conceived after 3 months (P=0.68) or from baseline to months 3 and 6 among those that did not conceive (P=0.99). Lastly, we lacked sun exposure data to determine dermal production of vitamin D. However, the latitudes of the 3 study locations were close and seasonal differences for the baseline collection of serum for 25(OH)D were minimal, and we used physical activity score as a proxy for sunlight exposure.

As is true in any observational study, it is possible that residual confounding or unmeasured confounders may be responsible for the association between vitamin D and pregnancy. In addition, intake of vitamin D could be an indirect indicator of other healthy lifestyle factors that improve the odds for pregnancy. One such example might be intake of certain nutrients and use of vitamin and mineral supplements that may be associated with fertility outcomes. However, overall dietary quality, as measured by the AHEI-P score did not differ between the two comparison vitamin D EAR groups, and we controlled for potential confounders related to selected dietary nutrients. The observed differences in intake of iron and folic acid among those who met or did not meet the vitamin D EAR were primarily contributed by vitamin and mineral supplement use. When examining food intake alone, there were no significant differences in iron and folic acid intakes between those who met or did not meet the vitamin D EAR. However, when comparing supplement intakes, those who met the vitamin D EAR were more likely to use supplements containing iron (95.2% vs. 38.8%, P<0.0001) and folic acid (96.4% vs. 55.1%, P<0.0001) than those who did not meet the vitamin D EAR. As such, the contribution of supplements led to higher total intakes of these nutrients from both foods and supplements combined. Our results show that there is a relationship between vitamin D intake requirements and conception. There may also be a relationship between vitamin D intake, intake of other nutrients and supplement use, and together may have an impact on healthier lifestyle patterns contributing to better fertility outcomes. However, it is unclear to what degree and how the intakes of these nutrients impact each other and subsequent conception outcomes. Further exploration in the contribution of these nutrients and vitamin and mineral supplement use should be investigated to better assess the complexities of their potential roles in residual confounding.

Education and income were significantly different among those who met and did not meet the vitamin D EAR (P=0.01 and p=0.002, respectively). However, in our study, income and education were significantly associated with supplement use. Among vitamin D supplement users, 81.8% had a household income ≥ $60,000 and 92.9% completed college or had an advanced degree. Those with a household income ≥ $60,000 had significantly higher vitamin D supplement intake (P=0.02) and subsequently higher total vitamin D intake (P=0.0007) than those with lower income. Furthermore, when comparing women who met or did not meet the vitamin D EAR from foods alone, there were no significant differences in both income (P=0.08) and education (P=0.44). These findings suggest that those who have higher income and education may have more knowledge on the benefits of supplement use and the ability to purchase supplements, and further strengthen the role that use of supplements has on contributing to increased intake as well as meeting the vitamin D EAR. The classification of meeting or not meeting the vitamin D EAR was based on total intake from both foods and supplement use. Therefore, income and education could be considered important upstream determinants of supplement use that could impact overall vitamin D intake and including them would lead to an over adjustment in the models. In addition, as discussed previously, higher vitamin D intake and use of vitamin D supplements could be associated with higher intakes of other nutrients and other markers of healthy lifestyle, some of which were examined in our models. Finally, among those who did and did not conceive a clinical pregnancy, there were no significant differences by income (p=0.49) or education (p=0.29). Since income and education were not independently related to the outcomes, they cannot be confounding variables. Therefore, we presented models that did not include income and education in this study.

Our study demonstrated an association between vitamin D intake and biomarker levels on conception. Those with intake at or above the recommended EAR and serum levels indicating sufficiency were more likely to conceive a clinical pregnancy even after controlling for baseline demographic and clinical characteristics. The association for vitamin D intake diminished after adjusting for total energy intake, total dietary and supplement energy adjusted intakes of polyunsaturated fat, iron and folic acid, and dietary quality. Given that 91% of the couples were unable to meet the vitamin D EAR with foods alone, a subgroup of the population that are not meeting these established levels might benefit from increased intake of vitamin D to help achieve the minimum recommended levels for adequacy. As there is no consensus for the definition of vitamin D deficiency among the various medical societies, and the appropriate levels to be utilized for assessment of clinical outcomes for at risk populations are debated, future studies in fertility should focus on defining optimal intake and biochemical levels of vitamin D to facilitate conception. In addition, intake and serum levels from both the male and female partners together should be explored to determine the impact of conceiving a clinical pregnancy, and to determine if certain groups would benefit from higher vitamin D supplementation to attain optimal intakes.

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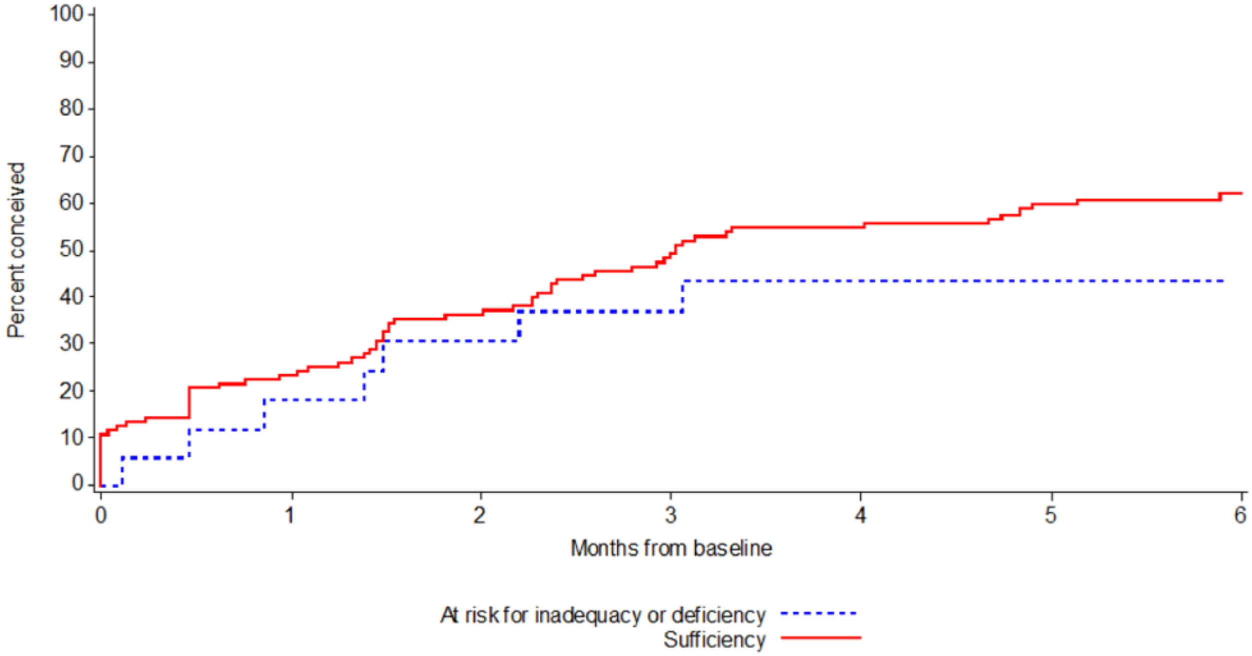

Figure 1.

Time to conception for all clinical pregnancies by serum 25(OH)D status among women, adjusted for age, race, body mass index category, alcohol consumption, smoking status, and physical activity score. At risk for inadequacy or deficiency is defined as serum 25-hydroxyvitamin D <50 nmol/L; sufficiency is defined as serum 25-hydroxyvitamin D ≥50 nmol/L.

Table 1

Demographic and lifestyle characteristics, and selected dietary intake measures of ISIS study participants who met or did not meet the EAR for energy-adjusted total vitamin D intake[a]

Characteristic	Number (%) or mean ± SD					
	Female (n=132)			Male (n=129)		
	Met vitamin D EAR (n=83)	Did not meet vitamin D EAR (n=49)	*P*-value[b]	Met vitamin D EAR (n=42)	Did not meet vitamin D EAR (n=90)	*P*-value[b]
Age at baseline (yrs)	29.9 ± 3.0	29.6 ± 3.5	0.58	31.6 ± 4.3	31.0 ± 4.5	0.44
Race			0.12			0.006
Caucasian	71 (85.6)	34 (69.4)		41 (100.0)	65 (73.8)	
Black	3 (3.6)	4 (8.2)		0 (0)	4 (4.6)	
Asian	7 (8.4)	9 (18.4)		0 (0)	14 (15.9)	
American Indian/Alaska Native	0 (0)	0 (0)		0 (0)	1 (1.1)	
Other/Multiracial	2 (2.4)	1 (2.0)		0 (0)	2 (2.3)	
Unknown	0 (0)	1 (2.0)		0 (0)	2 (2.3)	
Household income			0.002			0.39
< $60,000	14 (16.9)	21 (42.9)		7 (17.1)	27 (30.7)	
$60,000-$99,999	27 (32.5)	14 (28.6)		12 (29.3)	27 (30.7)	
$100,000-$1 39,999	24 (28.9)	4 (8.1)		12 (29.3)	16 (18.2)	
≥ $140,000	17 (20.5)	8 (16.3)		9 (21.9)	16 (18.2)	
Unknown	1 (1.2)	2 (4.1)		1 (2.4)	2 (2.2)	
Education			0.01			0.31
Completed high school	0 (0)	3 (6.1)		1 (2.4)	6 (6.8)	
Some college or Associate's degree	4 (4.8)	8 (13.3)		8 (19.5)	9 (10.2)	
Bachelor's degree	34 (41.0)	18 (36.8)		13 (31.7)	37 (42.1)	
Advanced degree	45 (54.2)	20 (40.8)		19 (46.3)	36 (40.9)	
BMI (kg/m^2)	24.1 ± 5.5	24.6 ± 5.1	0.58	26.6 ± 3.5	28.3 ± 6.6	0.04
BMI category[c]			0.67			0.01
Underweight	5 (6.0)	2 (4.1)		0 (0)	1 (1.1)	
Normal weight	51 (61.5)	26 (53.1)		13 (31.7)	31 (35.2)	
Overweight	18 (21.7)	13 (26.5)		23 (56.1)	27 (30.7)	
Obese	9 (10.8)	8 (16.3)		5 (12.2)	29 (33.0)	

Characteristic	Number (%) or mean ± SD					
	Female (n=132)			Male (n=129)		
	Met vitamin D EAR (n=83)	Did not meet vitamin D EAR (n=49)	*P*-value[b]	Met vitamin D EAR (n=42)	Did not meet vitamin D EAR (n=90)	*P*-value[b]
Smoking status			0.41			0.87
Current cigarettes or cigars	0 (0)	1 (2.0)		2 (5.0)	7 (8.0)	
Past cigarettes or cigars	16 (19.3)	11 (22.5)		15 (37.5)	35 (39.8)	
Never smoked	67 (80.7)	37 (75.5)		23 (57.5)	46 (52.2)	
Physical activity score[d]			0.47			0.70
Low	19 (22.9)	14 (28.6)		13 (31.7)	25 (28.4)	
High	64 (77.1)	35 (71.4)		28 (68.3)	63 (71.6)	
Alcohol use			0.34			0.75
None	13 (15.7)	13 (26.5)		6 (14.6)	14 (15.9)	
Occasional	26 (31.3)	17 (34.7)		10 (24.4)	23 (26.1)	
1 drink per week	14 (16.9)	4 (8.2)		3 (7.3)	13 (14.8)	
1 drink per day or several per week	29 (34.9)	14 (28.6)		20 (48.8)	35 (39.8)	
>1 drink per day	1 (1.2)	1 (2.0)		2 (4.9)	3 (3.4)	
Prior contraceptive use						
Oral contraceptives	75 (90.4)	40 (81.6)	0.15	---	--	---
Other hormonal contraceptives	2 (2.5)	0 (0)	0.53	---	--	---
Energy intake (kcals/d)	1695.0 ± 467.5	1639.4 ± 472.5	0.51	2348.7 ± 674.0	2058.1 ± 566.6	0.01
Total polyunsaturated fat intake from food and supplements (gm/d)	12.8 ± 3.6	12.6 ± 2.8	0.77	16.8 ± 4.4	16.8 ± 5.0	0.97
Total iron intake from food and supplements (mg/d)	38.9 ± 14.0	19.6 ± 11.8	<0.0001	23.7 ± 9.9	17.9 ± 7.4	0.0003
Total folic acid intake from food and supplements (μg/d)	1159.5 ± 586.3	636.0 ± 308.6	<0.0001	804.7 ± 280.3	518.4 ± 200.6	<0.0001
AHEI-P score[e]	72.7 ± 13.5	68.1 ± 13.8	0.06	74.9 ± 15.8	65.8 ± 14.7	0.002

[a]EAR, Estimated average requirement, namely, 10 μg/d

[b]Two-sample t-tests, chi-square and Fisher's Exact test were used to compare differences in demographic and lifestyle characteristics between those who met and did not meet the vitamin D EAR among the respective gender

[c]Categorized as underweight (BMI <18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (≥ 25 to <30 kg/m^2), and obese (≥ 30 kg/m^2)

[d]Categorized as low (< 1 hour/week of aerobic activity and < 2 hours/week of low intensity activity) and high (≥ 1 hour/week of aerobic activity and ≥ 2 hours/week of low intensity activity)

[e]AHEI-P dietary quality index based on a 130-point scale with a higher score indicative of a higher quality diet

Table 2

Energy-adjusted intake of vitamin D among ISIS study participants meeting and not meeting the EAR[a]

Intake	Overall			Met EAR[b]		Did not meet EAR[b]		P-value[c]
	n	Mean ± SD	% below EAR	n	Geometric mean (95% CI)	n	Geometric mean (95% CI)	
Female vitamin D intake (mg/d)								
Dietary intake	132	4.4 ± 3.1	95.5	83	4.4 (3.8-4.9)	49	3.0 (2.6-3.5)	0.0006
Dietary supplements[d]	99	16.1 ± 40.5	---	80	12.5 (10.8-14.4)	19	4.5 (3.7-5.5)	<0.0001
Total intake	132	16.5 ± 35.5	37.1	83	17.5 (15.6-19.7)	49	4.6 (3.8-5.4)	<0.0001
Male vitamin D intake (μg/d)								
Dietary intake	129	4.5 ± 2.9	93.8	41	4.8 (3.9-5.9)	88	3.5 (3.0-3.9)	0.005
Dietary supplements[d]	52	19.6 ± 60.2	---	35	13.6 (10.0-18.4)	17	3.3 (2.2-4.6)	<0.0001
Total intake	129	12.4 ± 39.6	68.2	41	17.9 (14.3-22.3)	88	4.1 (3.6-4.7)	<0.0001

[a]EAR, Estimated average requirement for total vitamin D intake, namely, 10 μg/d

[b]Comparison of meeting and not meeting EAR for total vitamin D intake from both food and supplements

[c]Two-sample t-test comparing intake among those who met and did not meet vitamin D EAR among the respective gender groups

[d]Supplement intake only included those participants who reported use of a vitamin D containing supplement on the days that the 24-hour dietary recalls were completed

Table 3

Clinical pregnancy and live birth outcomes according to energy-adjusted total dietary and supplement vitamin D intake and serum levels of women

Vitamin D	Clinical Pregnancy				Live Birth			
	Model 1AOR[a]		Model 2 AOR[b]		Model 1AOR[a]		Model 2 AOR[b]	
	OR (95% CI)	*P*-value[c]	OR (95% CI)	*P*-value[c]	OR (95% CI)	*P*-value[c]	OR (95% CI)	*P*-value[c]
Total dietary and supplement intake								
Did not meet EAR[d] (n=49)	Referent	0.038	Referent	0.42	Referent	0.042	Referent	0.26
Met EAR (n=83)	2.26 (1.05-4.86)		1.52 (0.56-4.15)		2.20 (1.03-4.69)		1.74 (0.66-4.60)	
Serum 25(OH)D[e]								
Inadequate or deficient (n=18)	Referent	0.039	Referent	0.11	Referent	0.053	Referent	0.11
Sufficient (n=112)	3.37 (1.06-10.70)		2.80 (0.81-9.71)	---	3.22 (0.99-10.55)		2.74 (0.78-9.56)	---

[a] Model 1 AOR: Adjusted odds ratio. For energy-adjusted total dietary and supplement intake, data were adjusted for age, race, body mass index category, alcohol consumption, and smoking status. For serum 25(OH)D, data were adjusted for age, race, body mass index category, alcohol consumption, smoking status, and physical activity score.

[b] Model 2 AOR: Adjusted odds ratio. For both energy-adjusted total dietary and supplement intake and serum 25(OH)D, data were adjusted for all covariates in Model 1 plus total energy intake, total dietary and supplement energy adjusted intakes of polyunsaturated fat, iron and folic acid, and AHEI-P score.

[c] Multiple logistic regression for adjusted odds ratio of achieving clinical pregnancy and live birth outcomes among those who met and did not meet vitamin D EAR, and those who had inadequate or deficient and sufficient serum 25(OH)D

[d] EAR, Estimated average requirement, namely, 10 μg/d

[e] 25(OH)D: 25-hydroxyvitamin D. Inadequate or deficient defined as 25(OH)D concentration <50 nmol/L. Sufficient defined as 25(OH)D concentration ≥50 nmol/L.